Appl. No. 10/738,454
Amdt. dated February 17, 2006
Reply to Office Action of August 23, 2005



Please replace the paragraph on page 6%, beginning with "Example 30", with the following replacement paragraph:

## **EXAMPLE 30**

## Selection of Mutagenized scFv-KJ16/Yeast by Fluorescence-Activated Cell Sorting

An *E. coli* mutator strain has been used to mutagenize an scFv for affinity maturation by phage display (Low et al., 1996). This approach was successful in identifying a mutant of scFv-4-4-20 with higher affinity for fluorescein using yeast display. A strength of this mutagenesis approach is its simplicity, requiring only *E. coli* transformation and cell growth. Furthermore, the *E. coli* mutator strain introduces mutations throughout the expression plasmid, and therefore does not bias changes to portions of the scFv believed to be important for determining binding characteristics. Whether this aspect of mutator strain mutagenesis is advantageous depends on the ability to identify key residues that might influence antigen binding, based on available structural information. Examination of published affinity maturation studies suggest that the location of such residues, generally in non-contact residues, is not yet predictable *a priori* (Hawkins et al., 1993, Patten et al., 1996, Schier et al., 1996, Thompson et al., 1996, Yang et al., 1995, Yelton et al., 1995).

Please replace the first paragraph on page 71 with the following replacement paragraph:

To apply this strategy to scFv-KJ16, the scFv-KJ16/Aga2 plasmid was propagated in the *E. coli* mutator strain XL1-Red (Strategene) for six cycles of growth. This procedure was predicted to introduce an average of two to three point mutations in the scFv coding sequence, based on a mutation rate per cycle of 1 in 2000 bps. The resultant plasmid preparation was transformed into yeast yielding a library size of approximately 3 X 10<sup>5</sup> transformants. In other work, larger libraries (10<sup>7</sup>) have been obtained by further optimization of transformation procedures and by pooling independent transformations. This number does not represent an upper size limit for library construction, as further efforts at optimization and scaleup could be straightforwardly applied.

Please replace the paragraph on page 71, line 13 through page 72, line 9 with the following replacement paragraph:

The mutagenized yeast library was subjected to four successive cycles of sorting and amplification, using a double stain for anti-c-myc antibody binding (FITC) and biotinylated-scTCR binding (PE). Biotinylated TCR was used at a 1:5000 dilution (~10 nM) that yielded just below the detectable threshold of binding by wt scFv-KJ16/yeast (Figure 13). The two channel fluorescence profiles of the mutated scFv-KJ16 sample after one sorting cycle (Figure 14A) and after four sorting cycles (Figure 14B) are shown. Cells that exhibited fluorescence above the diagonal window shown in Figure